

Mechanisms of enhancing soil fertility without obviously elevating global warming potential under an optimal rice straw incorporation rate in a paddy soil

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Abstract. The utility of rice straw as an organic fertilizer has been widely recognized as a promising approach to enhancing soil fertility. However, straw return is currently in a dilemma, as it may also provoke greenhouse gas (GHG) emissions, leading to serious environmental consequences. It is urgent to reveal the feasibility of straw incorporation regarding soil fertility improvement without notable increases in GHG emissions. Here, a soil microcosm experiment was conducted using paddy soils collected from a long-term rice straw incorporation field experiment. The treatments of the field experiment, namely CK, ST1, ST2, and ST3 corresponding to the straw application rates of 0, 50%, 100%, and 150% of the local rice straw yield, respectively, were continued in the microcosm experiment. The dynamics of GHG fluxes and concentrations in soils, and the variations in the abundances of soil microbial communities were systematically determined. The results indicated that ST1 treatment obviously improved soil fertility but did not induce significant elevation of global warming potential (GWP) with only 3% increase compared to CK. Although ST2 and ST3 treatments showed greater improvements in soil fertility, they significantly increased GWP by up to 151%. The minimal GWP increase under ST1 was mainly attributed to the significant reduction in N₂O emission and the slight rise in CH₄ emission compared to CK. The further investigations revealed that ST1 possessed the highest *nosZII* abundance and the lowest *nirS/nosZII* ratio, indicating its highest N₂O consumption ability. Meanwhile, its CH₄ production ability fluctuated around the soil CH₄ holding capacity (190 mmol·mol⁻¹), and most of the produced CH₄ was consumed by methanotrophs in soil. In conclusion, rice straw can be incorporated into paddy soils at a suitable application rate, which can effectively enhance soil fertility without inducing an additional warming effect.

30 1 Introduction

31 Rice cultivation not only provides staple food for nearly half of the global population, but also produces massive rice straw
32 that is estimated to be 800 to 1000 million tons·year⁻¹ globally (Yuan et al., 2021; Harun et al., 2022). Rice straw has long
33 been a valuable organic resource that can be incorporated into rice paddies to improve soil productivity (Huo et al., 2024;
34 Zhou et al., 2024). The incorporation can enhance soil carbon (C) sequestration (Chen et al., 2025; Li et al., 2025a). It was
35 estimated that about 15%–25% of the incorporated straw C was converted into soil organic carbon (SOC) (Liu et al., 2023b).
36 Straw return can also provide abundant available nutrients for rice plant growth, including nitrogen (N), phosphorus (P), and
37 potassium (K) (Yin et al., 2018; Van Hung et al., 2020; Wang et al., 2020). Thus, rice straw incorporation has been proposed
38 to be an important agronomic practice for sustainable rice agriculture.

39 However, rice straw return was also revealed to exacerbate greenhouse gas (GHG) emissions from paddy fields (Shi et al.,
40 2023; He et al., 2024). Rice cultivation contributes around 12% of global agricultural GHG emissions (FAO, 2020) with
41 straw incorporation being one of the key drivers. Numerous studies indicated that compared to straw removal, rice straw
42 incorporation substantially promoted methane (CH₄) emissions (Han et al., 2023; Song et al., 2024; Qin et al., 2025). The
43 increases in CH₄ emissions varied widely across observations, which approximately ranged from 23% to 1192% (Shen et al.,
44 2014; Jiang et al., 2019; Lee et al., 2020; Nan et al., 2022; Song et al., 2024). Straw incorporation may also elevate carbon
45 dioxide (CO₂) fluxes from rice paddies, for example, by 9% to 67% (Shen et al., 2014; Lee et al., 2020; Han et al., 2023;
46 Song et al., 2024). However, the effect of rice straw incorporation on nitrous oxide (N₂O) emissions from rice fields was
47 inconsistent and controversial across studies (Shen et al., 2014; Wang et al., 2015; Han et al., 2023; Qin et al., 2023). The
48 variations in N₂O emissions were about -73% to 137% in comparison with their controls (Shen et al., 2014; Wang et al.,
49 2015; Han et al., 2023; Qin et al., 2023). The variability in GHG emissions could be affected by several factors, such as soil
50 properties, agricultural management, and climate conditions, with the amount of straw incorporated potentially playing a
51 vital role. Optimizing the straw input rate was suggested to mitigate straw-induced GHG emissions (Shen et al., 2014; Chen
52 et al., 2018; Li et al., 2024). For instance, incorporating half of the harvested rice straw effectively reduced CH₄ emission by
53 48% relative to full straw incorporation (Shen et al., 2014). However, most studies focused either on the influence of straw
54 return on soil productivity (Huo et al., 2024; Zhou et al., 2024) or on GHG emissions (Shi et al., 2023; He et al., 2024),
55 rarely taking both into consideration to reveal the rational straw application. As a result, the balance and underlying
56 mechanisms between soil fertility and GHG emissions are still poorly understood, leaving no clear consensus on how much
57 straw should be incorporated appropriately. Hence, it is of high priority to investigate the suitable straw incorporation rate
58 and the mechanisms in improving soil fertility while not triggering substantial GHG emissions.

59 Soil GHG emissions are predominantly regulated by the microbially mediated production and consumption processes. CH₄
60 production and oxidation are catalyzed by methyl-coenzyme M reductase of methanogens and particulate methane
61 monooxygenase of methanotrophs, with their active subunits encoded by *mcrA* and *pmoA* genes, respectively (Gao et al.,
62 2020; Liu et al., 2025). The potential for N₂O production in paddy soils is closely associated with nitrite reduction, with

63 nitrite reductases encoded by *nirK* and *nirS* genes (Chen et al., 2010; Chen et al., 2024; Kong et al., 2024). N₂O reduction is
64 typically mediated by two distinct clades of N₂O reductase, encoded by *nosZI* and *nosZII* genes, respectively (Jones et al.,
65 2013; Hallin et al., 2018; Liu et al., 2023a; Yang et al., 2024). While straw incorporation generally enhanced CH₄ production,
66 its effects on CH₄ oxidation and N₂O production and reduction remain controversial. Some studies reported negligible effects
67 of straw application on *pmoA* abundance (Wang et al., 2018), but others found increases in *pmoA* abundance (Jiang et al.,
68 2019; Zhou et al., 2020a). For instance, long-term straw amendment increased *pmoA* abundance by 59% (Yang et al., 2022).
69 Likewise, straw incorporation led to inconsistent responses from N₂O-related genes (Wang et al., 2018; Wang et al., 2021).
70 The abundances of *nirK*, *nirS*, *nosZI*, and *nosZII* were reported to increase, decrease, or remain unchanged following straw
71 application (Wang et al., 2018). Taken together, the microbial mechanisms underlying GHG responses to straw
72 incorporation remain insufficiently understood, particularly how key functional microbial communities respond to different
73 straw application rates and thereby regulate GHG emissions.
74 Here, we hypothesized that there would be a moderate straw incorporation rate, which could well balance improving soil
75 fertility and minimizing the increase in GWP. Considering that field experiments can hardly avoid the influences of climate
76 and other factors, and the advantages of microcosm experiment including controlled environment and high operability in
77 frequent gas and soil sampling, a soil culture-microcosm experiment was employed to continue the field experiment with 5-
78 year rice straw incorporation at different rates. The incubation was set with flooding and drying periods. The dynamics of
79 GHG emissions and concentrations in soils were determined, and the variations in the abundance of soil microbial
80 communities involved in GHG production and consumption were measured. This study aimed to reveal the mechanisms of
81 boosting soil fertility without elevating GWP under an optimal rice straw incorporation rate.

82 **2 Materials and methods**

83 **2.1 Site description and experimental design**

84 A long-term straw return field experiment was initiated in 2017, which was located at the Taoyuan Agroecosystem Research
85 Station (28°55' N, 111°26' E), Hunan, China. The region has a subtropical monsoon climate with a mean annual rainfall of
86 1440 mm and a mean annual temperature of 16.5°C. The soil was derived from quaternary red clay and classified as Ultisol
87 according to USDA soil taxonomy. The experiment contained various rates of rice straw amendments with three replications
88 in a randomized block design. Each plot was 30 m² (7.5 m × 4 m) and the cropping was single rice. Rice straw cut into 10
89 cm segments was evenly spread on the surface of the corresponding plots and turned over into the plow layer (0–20 cm) in
90 each early May. Urea, superphosphate, and potassium chloride were annually applied to all treatments at 112.60 kg N·ha⁻¹,
91 46.33 kg P·ha⁻¹, and 135.46 kg K·ha⁻¹, respectively. All treatments were subjected to the same water management. During
92 the rice season, all plots were continuously flooded except for two drying events. The first drying event occurred at the end
93 of the tillering stage and lasted for one to two weeks. The second drying event happened one week before harvest. In the
94 fallow season, all plots remained unirrigated.

95 2.2 Soil and straw sampling and preparation

96 Soils (0–20 cm) were separately taken from CK, ST1, ST2, and ST3 treatments of the field experiment in May 2022 before
97 straw application. The corresponding straw incorporation rates were 0, 3750, 7500, and 11250 kg·ha⁻¹, representing 0, 50%,
98 100%, and 150% of the local rice straw yield, respectively. The soil from each treatment was collected by taking five points
99 following an “S” pattern in each plot and mixed, and then transported to the lab. The fresh soils were directly flooded and
100 homogenized into slurries to avoid the impact of air-drying on microbial communities. Plant residues and gravel were
101 removed by sieving the slurries through a 2 mm mesh, and the resulting soil slurries were pre-incubated under flooding at
102 room temperature for 30 days. The soil physicochemical properties were shown in Table 1.
103 Rice straw was prepared by collecting it from the field in October 2021, followed by air-drying and grinding (< 1 mm) for
104 the microcosm experiment. The sieved straw can be evenly distributed and contacted with soil particles, generating similar
105 decomposition speeds among the pots of each treatment. The rice straw contained 44.14% of C, 0.817% of N, 2.34% of K,
106 and 0.143% of P.

107 2.3 Microcosm experiment

108 The incubation pots were prepared by sealing the bottoms of PVC cylinders (height 15 cm, diameter 15.5 cm). The pots for
109 gas sampling were further equipped as follows (Fig.S1). For gas flux sampling, a hollow rectangular groove (deep 2 cm) was
110 fixed on the top fringe of each pot for water sealing during sampling. The gas flux samples were collected using a static
111 chamber (height 29.5 cm, diameter 19.5 cm) fitted with a small electric fan at the inner top. For soil profile gas sampling,
112 two holes (diameter 1.7 cm) across the cylinder were drilled at positions of 5 cm from the bottom, filled with a gas-
113 permeable silicone tube (length 15.5 cm, inner diameter 1.2 cm) closed at both ends with silicone septa. One end of the tube
114 was connected with a three-way stopcock outside the cylinder via a stainless steel tube (diameter 0.2 cm) through the septum.
115 The drilled holes in the cylinder wall were then sealed.
116 The pre-incubated soils drained of surface water from the different field treatments were separately filled into the pots. Each
117 pot was filled with wet soil to 10 cm in height containing 1.27 kg of dry soil. The straw addition amounts in the incubation
118 were 0, 2.00, 4.00, and 6.00 g straw·kg⁻¹ dry soil for CK, ST1, ST2, and ST3 treatments, respectively, which were converted
119 from the straw incorporation rates of the field experiment. Each treatment had eight pots: three for gas sampling, three for
120 soil sampling, and two for replacing sampling-induced soil columns to minimize gas exchange from the sampled holes.
121 Urea was prepared as a stock solution (12.70 g N·L⁻¹), and 10 mL of this solution was added to each pot, equivalent to 100
122 mg N·kg⁻¹ dry soil. After urea and straw were applied, the soil was thoroughly mixed immediately to ensure that the straw
123 and N fertilizer can be uniformly distributed in the soil. Then, deionized water was added to form a 3 cm surface water layer.
124 The prepared microcosms were randomly positioned and incubated in the dark at 30°C for 91 days (28-day flooding and 63-
125 day drying). This incubation period was based on our pre-experiment to cover both CH₄ and N₂O emission peaks. During

126 flooding, a 3 cm top water layer was maintained with deionized water. The drying process was carried out by removing the
127 top water from the microcosm with a syringe on day 28 and then drying by natural evaporation in the room.

128 **2.4 Gas and soil sampling**

129 Gas samples for GHG fluxes and soil profile concentrations were taken between 9:00 and 11:00 AM every 1–3 days. At each
130 sampling event, after the groove was filled with water and covered by the chamber, 30 mL of headspace gas was taken with
131 a syringe separately at 0 and 60 min from each gas sampling pot (Wang et al., 2017; Zhou et al., 2020b). The electric fan was
132 running during the sampling period. Immediately after collecting all headspace gas samples, 1 mL of soil gas was taken from
133 each buried silicone tube, with an equal volume of helium injected back. The gas from the silicone tube was used to
134 determine the soil CO₂, CH₄, and N₂O concentrations. All the gas samples were stored in 12 mL pre-evacuated exetainer
135 vials (Labco, UK).

136 Soil samples (0–5 cm) were taken every two weeks via a modified syringe sampler with its head cut off. At each sampling
137 event, three cores (diameter 1.5 cm) were sampled from each pot and mixed, weighing about 35 g wet soil. During flooding,
138 the soil samples were collected directly without removing the overlying water to minimize the disturbance (Cai et al., 2022;
139 Cai et al., 2025). Before starting soil sampling, placing the front end of the syringe sampler as close as possible to the soil
140 surface effectively reduced the collection of the overlying water. A part of the sample was used for soil moisture
141 measurement, and the rest was flash-frozen in liquid nitrogen and stored at -80°C for molecular analysis. After sampling, the
142 holes were immediately refilled with the cores taken from the soil replacement pots.

143 **2.5 Analysis of gas concentrations and soil properties**

144 Gas samples were analyzed using a gas chromatography (7890A, Agilent, USA) equipped with an electron capture detector
145 for N₂O and a flame ionization detector for CH₄ and CO₂. Gas fluxes were calculated based on the Ideal Gas Law (Eq. 1 and
146 Eq. 2), and cumulative emissions by the Trapezoidal Rule (Eq. 3). Global warming potential (GWP) was calculated using
147 cumulative emissions (Eq. 4).

$$148 \quad F = \frac{\Delta m}{A \times \Delta t}, \quad (1)$$

149 Where F is the gas flux ($\mu\text{g} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$); Δm is the mass of gas collected in a single sampling event (μg); A is the soil surface
150 area covered by the static chamber (m^2); Δt is the time interval after chamber closure (h).

$$151 \quad \Delta m = M \times \Delta n = M \times \frac{pV \times \Delta c}{RT}, \quad (2)$$

152 Where M is the molar mass of gas ($\text{g} \cdot \text{mol}^{-1}$); Δn is the amount of substance of gas collected in a single sampling event (mol);
153 p is the atmospheric pressure (Pa); V is the active volume of the static chamber during sampling (m^3); Δc is the gas
154 concentration difference (ppm); R is the gas constant ($8.314 \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$); T is the temperature during sampling (K).

$$155 \quad CE = \sum_{i=1}^n \frac{F_{i+1} + F_i}{1000} \times (t_{i+1} - t_i) \times 24 \times \frac{1}{2}, \quad (3)$$

Where CE is the gas cumulative emission ($\text{g}\cdot\text{m}^{-2}$); F is the gas flux ($\mu\text{g}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$); 1000 in the denominator is to transform μg to g ; t is the sampling time (d); i and $i+1$ represent the i^{th} and $(i+1)^{\text{th}}$ sampling events, respectively; 24 is the transform factor from day to hour; n is the total number of sampling events.

$$GWP = CE_{\text{CO}_2} + 27.9 \times CE_{\text{CH}_4} + 273 \times CE_{\text{N}_2\text{O}}, \quad (4)$$

Where the unit of GWP is $\text{g CO}_2 \text{ equivalent}\cdot\text{m}^{-2}$. CE_{CO_2} , CE_{CH_4} , and $CE_{\text{N}_2\text{O}}$ represent the cumulative emissions ($\text{g}\cdot\text{m}^{-2}$) of CO_2 , CH_4 , and N_2O , respectively. The factors 27.9 and 273 are the latest-updated 100-year global warming potentials for CH_4 and N_2O , respectively (Forster et al., 2021).

Soil properties were measured by standard protocols (Bao, 2000). Soil pH was determined using soil slurry at a soil–water ratio of 1:2.5 by a pH meter (FE28–Standard, Mettler Toledo, Switzerland). Soil bulk density (BD) was measured by the ring knife method. Soil cation exchange capacity (CEC) was extracted by 1.66 $\text{cm Co}(\text{NH}_3)_6\text{Cl}_3$ at a soil–extractant ratio of 7:100. Olsen P was extracted by 0.5 M NaHCO_3 at a soil–extractant ratio of 1:20. Soil CEC and Olsen P were quantified colorimetrically via a continuous–flow automatic analyzer (AutoAnalyzer 3, SEAL Analytic, Germany). Available N (AvN) was determined by the alkaline diffusion method. Available K (AvK) was extracted by 1 M NH_4OAc at a soil–extractant ratio of 1:10 and analyzed via an atomic absorption spectrometer (novAA350, Analytic Jena, Germany). Soil available manganese (AvMn), copper (AvCu), and zinc (AvZn) were simultaneously extracted by 5 mM $\text{DTPA}\text{--}\text{CaCl}_2\text{--TEA}$ at a soil–extractant ratio of 1:2 and determined by an inductively coupled plasma emission spectrometer (ICP–OES 5110, Agilent, USA). Soil total C (TC) and total N (TN) were analyzed by combustion using an elemental analyzer (Vario MAX cube, Elementar, Germany). Soil total P (TP) and total K (TK) were measured by the ICP–OES after digestion by $\text{HNO}_3\text{--HClO}_4\text{--HF}$.

2.6 DNA extraction and real-time quantitative PCR

Soil DNA was extracted manually as previously described (Chen et al., 2010). Each sample was performed in triplicate. The quality and concentration of DNA were measured by a NanoDrop ND–1000 spectrophotometer (Thermo Scientific, USA). The abundances of bacterial 16S rRNA, fungal 18S rRNA, *mcrA*, *pmoA*, *nirK*, *nirS*, *nosZI*, and *nosZII* were determined by real-time quantitative PCR (qPCR). The qPCR primers and thermal programs were shown in Table S1. All qPCR reactions were conducted in triplicate on 384-well plates by a LightCycler 480II (Roche, Switzerland). Each reaction (10 μL) contained 1 μL of template DNA (5 $\text{ng}\cdot\mu\text{L}^{-1}$), 5 μL of SYBR Green Pro Taq HS Premix (Accurate Biotechnology, China), and 0.3–1.2 μL of each primer (10 μM). Negative controls were included in each plate by replacing the template DNA with sterile H_2O . Standard curves were prepared by ten-fold serial dilutions of target gene-carrying plasmids. The amplification efficiency was 87%–88% for *nosZII* and 90%–102% for other genes.

2.7 Statistical analysis

Statistical tests were performed in IBM SPSS Statistics 25 (NY, USA) and OriginPro 2026 (OriginLab Corporation, MA, USA). Homoscedasticity ($\alpha = 0.05$) was checked with Levene’s test. Significant differences between treatments ($\alpha = 0.05$)

188 were tested by one-way analysis of variance (ANOVA), followed by Tukey’s Honestly Significant Difference (HSD) test
 189 when data were homoscedastic or Games–Howell (GH) test when data were heteroscedastic. Spearman correlation analysis
 190 was conducted using corrplot package in R v4.5.3 ($\alpha = 0.05, 0.01, \text{ or } 0.001$).

191 **3 Results**

192 **3.1 Influence of straw incorporation on soil fertility**

193 Soil physicochemical characteristics were clearly affected by continuous straw incorporation at various rates for five years
 194 (Table 1). The results indicated that soil BD was negatively and significantly correlated with the rice straw incorporation rate
 195 ($p < 0.05$), ~~suggesting the physical character was improved. Concerning the detected soil chemical properties, except for TP,~~
 196 ~~TK, and Olsen P, which were not clearly influenced by straw incorporation, the rest properties~~and soil pH, CEC ($p < 0.05$),
 197 AvN, AvK ($p < 0.001$), AvMn, AvCu, AvZn ($p < 0.05$), TC, and TN were positively related to the straw application rate.
 198 ~~Among them, the variations of CEC ($p < 0.05$), AvK ($p < 0.001$), and AvZn ($p < 0.05$) were significantly affected. Although~~
 199 ~~most of the characteristics were not statistically significant among treatments under field conditions. When~~when compared
 200 with CK, even the least amount of straw input (ST1) induced the decrease of BD by 2.76% and the increases of CEC, TC,
 201 TN, AvN, Olsen P, and AvK by 3.13%, 4.19%, 2.58%, 6.83%, 15.09%, and 38.52%, respectively. ST1 treatment also
 202 resulted in obvious increases in soil micronutrient concentrations in comparison with CK, with the contents of AvMn, AvCu,
 203 and AvZn elevated by 18.48%, 4.57%, and 8.09%, respectively. These data suggested an improving trend in soil fertility
 204 resulting from rice straw incorporation.

205 **Table 1: Soil properties after five-year straw incorporation at different rates**

	pH (H ₂ O) (1:2.5)	BD g·cm ⁻³	CEC cmol ⁺ ·kg ⁻¹	AvN mg·kg ⁻¹	Olsen P mg·kg ⁻¹	AvK mg·kg ⁻¹	AvMn mg·kg ⁻¹	AvCu mg·kg ⁻¹	AvZn mg·kg ⁻¹	TC g·kg ⁻¹	TN g·kg ⁻¹	TP g·kg ⁻¹	TK g·kg ⁻¹
CK	4.90± 0.03 a	1.45± 0.03 a	10.85± 0.15 a	192.79± 5.38 a	13.19± 1.76 a	51.09± 2.70 c	8.28± 0.22 a	1.75± 0.10 a	1.36± 0.07 a	21.74± 0.46 a	2.057± 0.04 a	0.618± 0.03 a	12.16± 0.08 a
ST1	4.98± 0.05 a	1.41± 0.01 a	11.19± 0.23 a	205.96± 3.59 a	15.18± 1.68 a	70.77± 3.62 bc	9.81± 1.50 a	1.83± 0.09 a	1.47± 0.10 a	22.65± 0.59 a	2.110± 0.05 a	0.657± 0.02 a	12.01± 0.11 a
ST2	4.93± 0.02 a	1.34± 0.07 a	11.25± 0.20 a	212.07± 8.15 a	12.57± 1.33 a	82.35± 5.01 ab	11.65± 2.30 a	1.94± 0.07 a	1.70± 0.07 a	23.52± 0.70 a	2.197± 0.07 a	0.610± 0.03 a	11.95± 0.19 a
ST3	5.02± 0.07 a	1.31± 0.03 a	11.45± 0.26 a	209.22± 11.70 a	16.06± 2.22 a	105.36± 11.01 a	16.69± 5.97 a	1.88± 0.05 a	1.76± 0.18 a	23.71± 1.45 a	2.160± 0.12 a	0.648± 0.04 a	12.04± 0.03 a
r	0.45	-0.67*	0.63*	0.45	0.17	0.93***	0.48	0.30	0.67*	0.50	0.39	0.11	-0.26

206 These soils were collected from the plots of a long-term field experiment for the microcosm experiment. CK, ST1, ST2, and
 207 ST3 received 0, 50%, 100%, and 150% of the local harvested rice straw, respectively. Different lower-case letters indicate
 208 significant differences between treatments ($\alpha = 0.05$). Data are given in average $\pm$ standard error ($n = 3$). The Spearman
 209 correlation coefficient (r) between soil properties and straw rate is presented in the last row. ~~S,~~ and significant values are
 210 shown in bold. *, $\alpha = 0.05$; **, $\alpha = 0.01$; ***, $\alpha = 0.001$. BD, bulk density; CEC, cation exchange capacity; AvN, available
 211 nitrogen; Olsen P, available phosphorus; AvK, available potassium; AvMn, available manganese; AvCu, available copper;
 212 AvZn, available zinc; TC, total carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium.

213 3.2 ~~CO₂, CH₄, and N₂O emissions~~Influence of straw incorporation on GHG emissions and global warming potential

214 Greenhouse gas emissions were obviously influenced by straw incorporation (Fig.1, Table 2). Generally, the CO₂ flux was
215 positively related to the straw incorporation rate throughout the incubation (Fig.1a, Table 3). Compared to CK, ST1 caused a
216 significant increase in the cumulative CO₂ emission by 66% ($p < 0.05$, Table 2). With the straw amount increasing, the
217 cumulative CO₂ emissions of ST2 and ST3 treatments rose to 2.2 and 2.5 times those of CK ($p < 0.05$), respectively. A
218 significant difference was also detected between ST2 and ST3 ($p < 0.05$).

219 The CH₄ fluxes occurred mainly during flooding (day 0–day 28), and partly during the transition time from flooding to
220 drying (day 29–day 56, Fig.1b, Table S2). It was observed that CK emitted minimal CH₄, while ST1 emitted a slightly
221 higher CH₄ than CK. Further increasing the straw rate induced significant elevation of CH₄ fluxes. The more straw input, the
222 more CH₄ was emitted. The cumulative CH₄ emission of ST1 was only 4.41 g·m⁻², while the cumulative CH₄ emissions of
223 ST2 and ST3 were approximately 4 and 8 times higher than those of ST1 ($p < 0.05$), respectively, reaching about 23 and 38
224 g·m⁻² (Table 2).

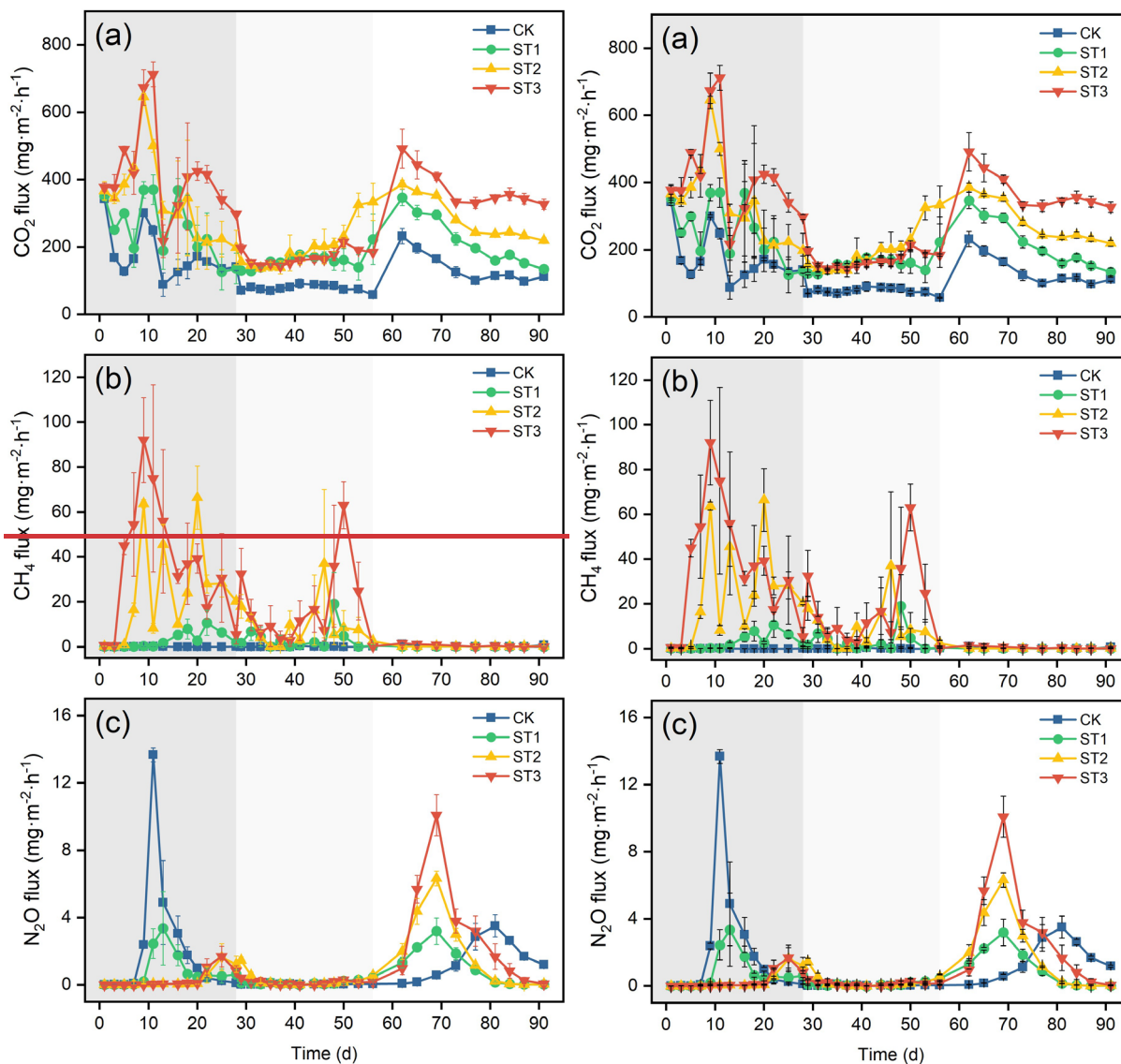


Figure 1: Dynamics of GHG fluxes during the incubation. (a) CO₂; (b) CH₄; (c) N₂O. CK, ST1, ST2, and ST3 received 0, 50%, 100%, and 150% of the local harvested rice straw, respectively. Data are given in average $\pm$ standard error (n = 3). Grey, lighter grey, and white areas indicate the flooding, the early drained ~~period~~, and the late drained period, respectively.

Table 2: Cumulative emissions and global warming potential

<u>Treatment</u>	Cumulative emissions			Global warming potential
	CO ₂ (g·m ⁻²)	CH ₄ (g·m ⁻²)	N ₂ O (g·m ⁻²)	(g CO ₂ equivalent·m ⁻²)
CK	280.19 $\pm$ 4.37 d	0.36 $\pm$ 0.02 d	2.61 $\pm$ 0.08 ab	1003.49 $\pm$ 24.74 c

ST1	465.65 ± 16.07 c	4.41 ± 0.52 c	1.63 ± 0.11 c	1034.95 ± 48.01 c
ST2	605.91 ± 26.84 b	23.22 ± 0.93 b	2.04 ± 0.17 bc	1810.05 ± 56.46 b
ST3	695.71 ± 17.59 a	38.01 ± 0.35 a	2.80 ± 0.20 a	2521.49 ± 80.01 a

231 CK, ST1, ST2, and ST3 received 0, 50%, 100%, and 150% of the local harvested rice straw, respectively. Different lower-
232 case letters indicate significant differences between treatments ($\alpha = 0.05$). Data are given in average $\pm$ standard error (n = 3).

233 **Table 3: Spearman correlation between straw rate, cumulative GHG emissions, and microbial gene abundances**

	CO ₂	CH ₄	N ₂ O	<u>Bacteri</u> <u>a16S</u> <u>rRNA</u>	<u>Fungi1</u> <u>8S</u> <u>rRNA</u>	<i>mcrA</i>	<i>pmoA</i>	<i>nirK</i>	<i>nirS</i>	<i>nosZI</i>	<i>nosZII</i>
Straw rate	0.97***	0.97***	0.22	0.91***	0.97***	0.97***	0.78**	0.78**	0.82**	0.91***	0.28
CO ₂				0.87***	0.95***	0.96***	0.76**	0.76**	0.79**	0.89***	0.30
CH ₄				0.85***	0.95***	0.96***	0.78**	0.74**	0.75**	0.87***	0.25
N ₂ O				0.26	0.21	0.28	0.17	0.64*	0.13	0.16	-0.50

234 CO₂, CH₄, and N₂O represent the corresponding cumulative emissions. Significant values are shown in bold. *, $\alpha = 0.05$; **, $\alpha = 0.01$; ***, $\alpha = 0.001$.

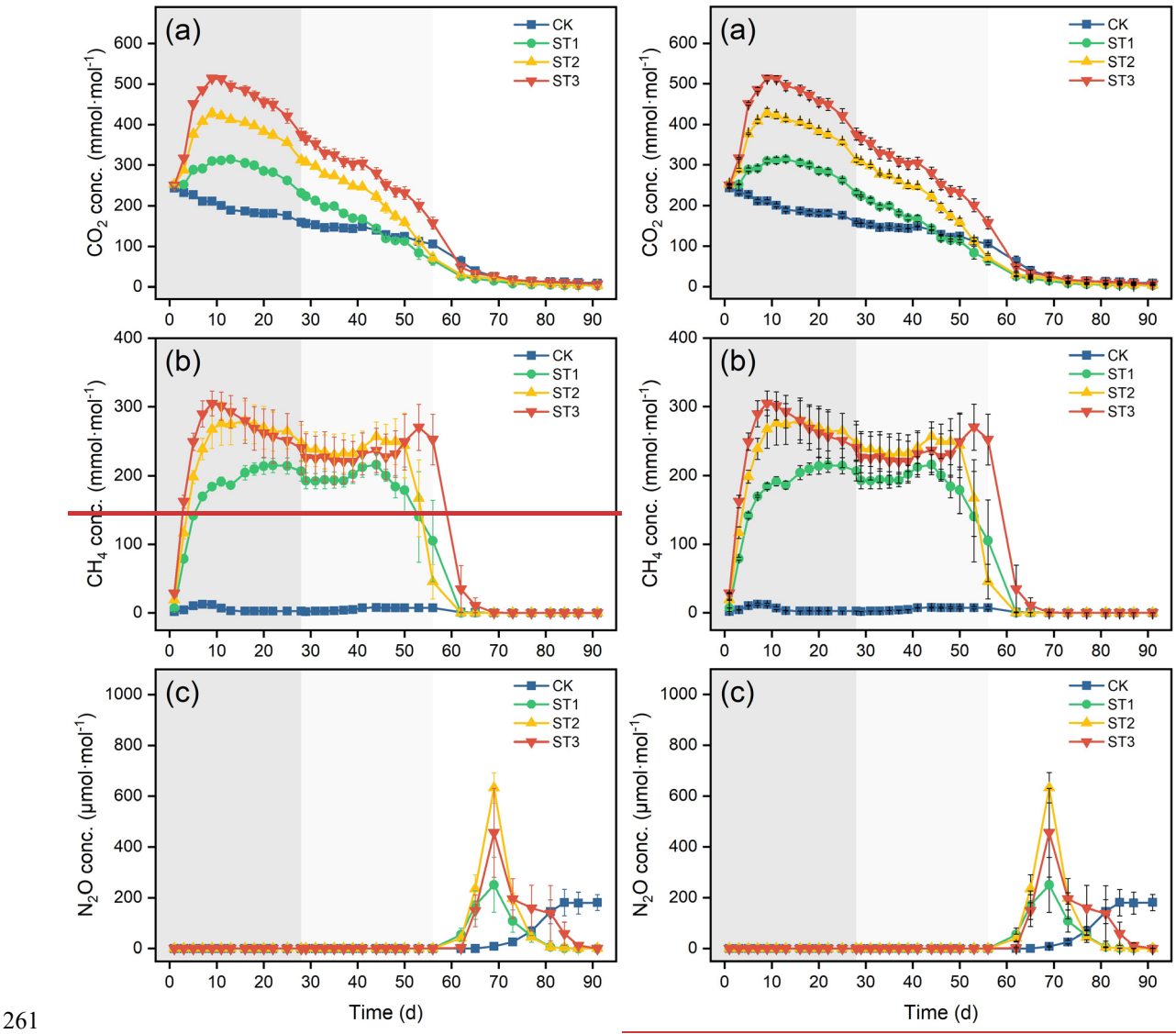
236 The dynamics of N₂O emission exhibited two distinct types of peaks, with one occurring shortly-type peaking as early as day
237 11 after fertilization and the other during the late drying period (Fig.1c). However, the variation patterns of the two peaks
238 differed markedly both among treatments and between periods. During the first peak, CK emitted most N₂O, followed by
239 ST1, while the N₂O emitted from ST2 and ST3 were negligible. However, this order reversed in the latter drying period,
240 where the highest N₂O flux was from ST3, followed by ST2, whereas the N₂O fluxes of ST1 and CK were similarly low,
241 despite that the peak of CK appeared about 10 days late. The cumulative N₂O emission indicated that ST1 emitted
242 significantly lower N₂O than CK ($p < 0.05$) with only 1.63 g·m⁻² (Table 2). Meanwhile, ST2 also generated about 22%
243 lower N₂O than CK, while the treatment receiving the highest amount of straw (ST3) possessed a similar cumulative N₂O
244 emission to CK.

245 Overall, ST1 displayed a slightly higher GWP than CK without a significant difference, but both ST2 and ST3 showed
246 significantly higher GWPs than CK ($p < 0.05$).

247 3.3 Dynamics of CO₂, CH₄, and N₂O concentrations in soils

248 The soil concentrations of the three GHGs were deeply affected by the amendments of rice straw but exhibited distinct
249 patterns (Fig.2). Straw incorporation boosted the soil CO₂ concentration compared to the continuously declining CK, with
250 the peaking time of straw-amended treatments occurring around 10 days of the incubation (Fig.2a). The outstanding
251 characteristic was that the increases were proportional to the straw incorporation rates. For example, at the peak, the
252 increments in ST1, ST2, and ST3 relative to CK were approximately 50%, 100%, and 150%, respectively.

253 The soil CH₄ concentrations indicated that straw incorporation resulted in sharp increases since day 3 (Fig.2b). On average,
 254 the CH₄ concentrations of ST1, ST2, and ST3 were about 45, 57, and 58 times that of CK between day 1 and day 56. It was
 255 also observed that the CH₄ concentrations of ST1 fluctuated around 200 mmol·mol⁻¹. ST2 and ST3 further promoted soil
 256 CH₄ concentrations but there was no obvious differences between them.
 257 The soil N₂O concentrations were only detectable between days 62 and 87 under drying phase (Fig.2c). The unique feature
 258 was that the N₂O contents of CK and ST1 were quite similar despite the peaking time in CK was delayed. It was observed
 259 that the N₂O concentration of ST2 was almost two times that of ST1. Although the peak of ST3 was not as high as that of
 260 ST2, ST3 maintained high N₂O concentrations for longer in the soil.



262 **Figure 2: Dynamics of GHG concentrations in soils during the incubation. (a) CO₂; (b) CH₄; (c) N₂O. CK, ST1, ST2,**
 263 **and ST3 received 0, 50%, 100%, and 150% of the local harvested rice straw, respectively.** The abbreviation *conc.*
 264 means concentration. Data are given in average $\pm$ standard error (n = 3). Grey, lighter grey, and white areas indicate
 265 the flooding, the early drained ~~period~~, and the late drained period, respectively.

266 3.4 Variations of microbial abundance

267 3.4.1 The abundance of bacteria and fungi

268 ~~In general, the abundance of both bacterial 16S rRNA and fungal 18S rRNA followed the order of ST3 > ST2 > ST1 > CK~~
 269 ~~(Fig.3). The Across the six sampling days, the~~ average copy numbers of 16S rRNA were $3.92E+ \times 10^{12}$, $4.50E+ \times 10^{12}$,
 270 $4.76E+ \times 10^{12}$, and $5.71E+ \times 10^{12}$ per g dry soil for CK, ST1, ST2, and ST3, respectively (Fig. 3). The ~~average abundances~~
 271 ~~of corresponding values for~~ 18S rRNA were $2.04E+ \times 10^9$, $2.78E+ \times 10^9$, $3.03E+ \times 10^9$, and $3.63E+ \times 10^9$ per g dry soil,
 272 respectively. ~~The bacterial and fungal population sizes exhibited similar temporal dynamics during the incubation. They~~
 273 ~~were obviously affected by straw incorporation on day 14 under flooding, when ST2 and ST3 induced significantly higher~~
 274 ~~abundances of bacteria and fungi than CK, but no significant differences were detected between ST1 and CK. Afterwards,~~
 275 ~~the populations of bacteria and fungi fluctuated among treatments until day 56 during the early drained phase, on which the~~
 276 ~~influence of straw incorporation on the microbial abundances appeared again and ST3 showed significantly higher~~
 277 ~~population sizes of bacteria and fungi than CK. During the late drained period on days 70 and 84, this influence continued,~~
 278 ~~where ST3 possessed significantly larger bacterial populations than CK, and the three straw-amended treatments resulted in~~
 279 ~~significantly higher fungal abundances than CK.~~

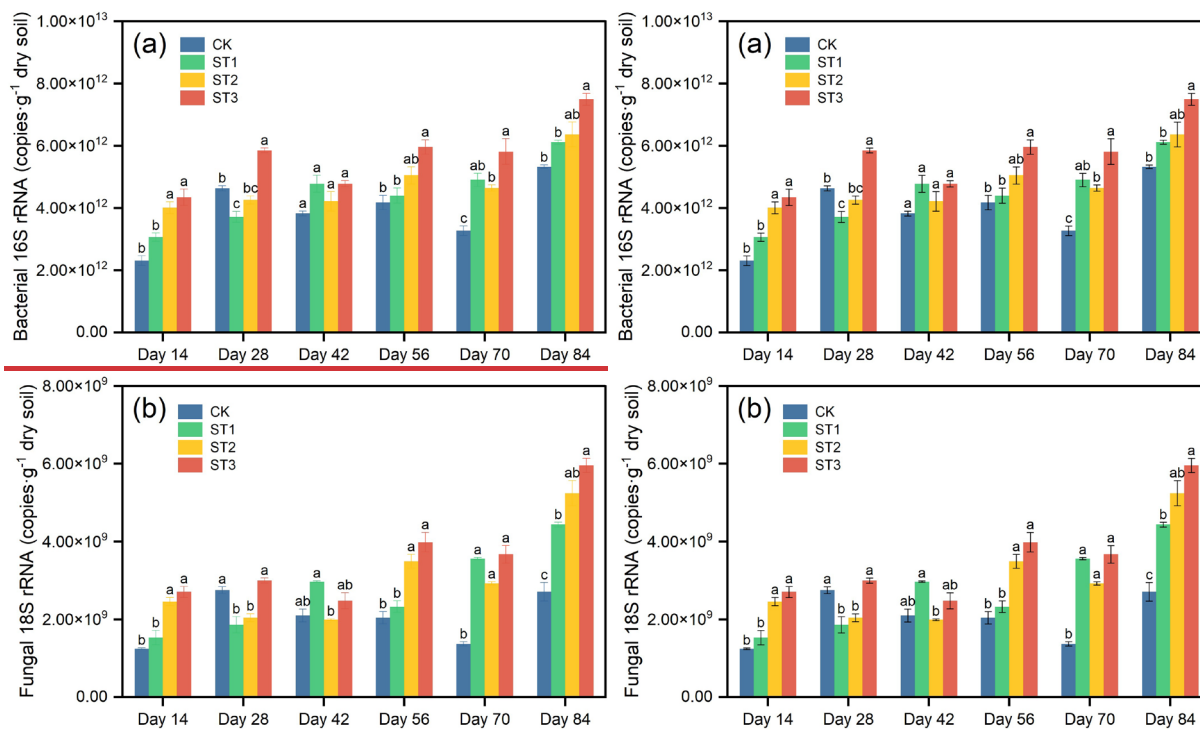


Figure 3: Dynamics of bacterial 16S rRNA abundance (a) and fungal 18S rRNA abundance (b) during the incubation. CK, ST1, ST2, and ST3 received 0, 50%, 100%, and 150% of the local harvested rice straw, respectively. Different lower-case letters denote significant differences between treatments ($\alpha = 0.05$). Data are given in average $\pm$ standard error ($n = 3$). Days 14 and 28 correspond to the flooding period, days 42 and 56 to the early drained period, and days 70 and 84 to the late drained period, respectively.

3.4.2 The abundance of methanogens and methanotrophs

The copy number of *mcrA* was markedly affected by straw incorporation and positively related to the straw input rate (Fig.4a, Table 3). The average *mcrA* abundance during the CH₄ fluxing period between day 14 and day 56 was significantly and positively related to the straw input rate. In comparison with CK, the least average increase occurred in ST1 with 32%, while the highest happened in ST3 with 129%.

For *pmoA*, although straw incorporation also clearly promoted its copy number, the treatment with the lowest amount of straw (ST1) induced a substantial increase in methanotrophs compared to CK by approximately 81% on average between day 14 and day 56 (Fig.4b). Further increasing the straw amount failed to cause significant elevations in comparison with ST1 (Fig.4b).

When concerning *mcrA/pmoA*, the average ratio of ST1 between day 14 and day 56 was quite similar to that of CK, while the ratios of ST2 and ST3 were significantly higher than that of CK (Fig.4c).

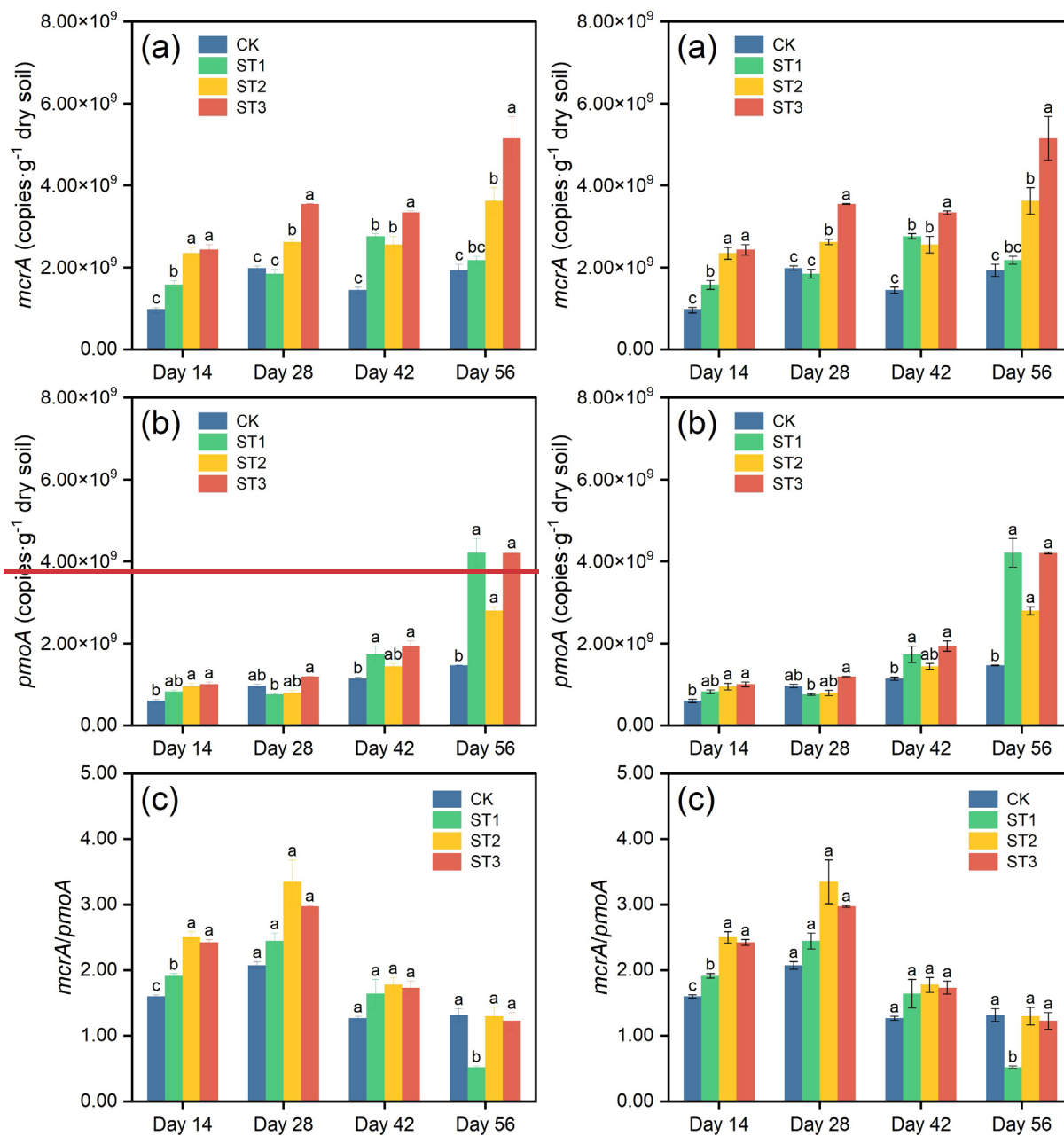


Figure 4: Dynamics of *mcrA* abundance (a), *pmoA* abundance (b), and *mcrA/pmoA* ratio (c) during the CH₄ fluxing period. CK, ST1, ST2, and ST3 received 0, 50%, 100%, and 150% of the local harvested rice straw, respectively. Different lower-case letters denote significant differences between treatments ($\alpha = 0.05$). Data are given in average $\pm$ standard error ($n = 3$). Days 14 and 28 correspond to the flooding period, and days 42 and 56 to the early drained period, respectively.

303 3.4.3 The abundance of N₂O-producing and N₂O-reducing communities

304 The population size of *nirS* was relatively stable, and the variations among treatments were negligible under flooding
305 (Fig.5a). In contrast, under drying, the copy number of *nirS* increased and was clearly affected by straw incorporation. All
306 the straw-amended treatments outstandingly increased *nirS* abundance compared to CK. Among the treatments, ST3
307 possessed the highest *nirS* copy numbers, while ST2 and ST1 had similarly lower abundances. By comparison, the
308 abundances of *nirK* declined after drainage and were less affected by straw incorporation (Fig. S2a) than those of *nirS*
309 (except ST3, Fig. S2).

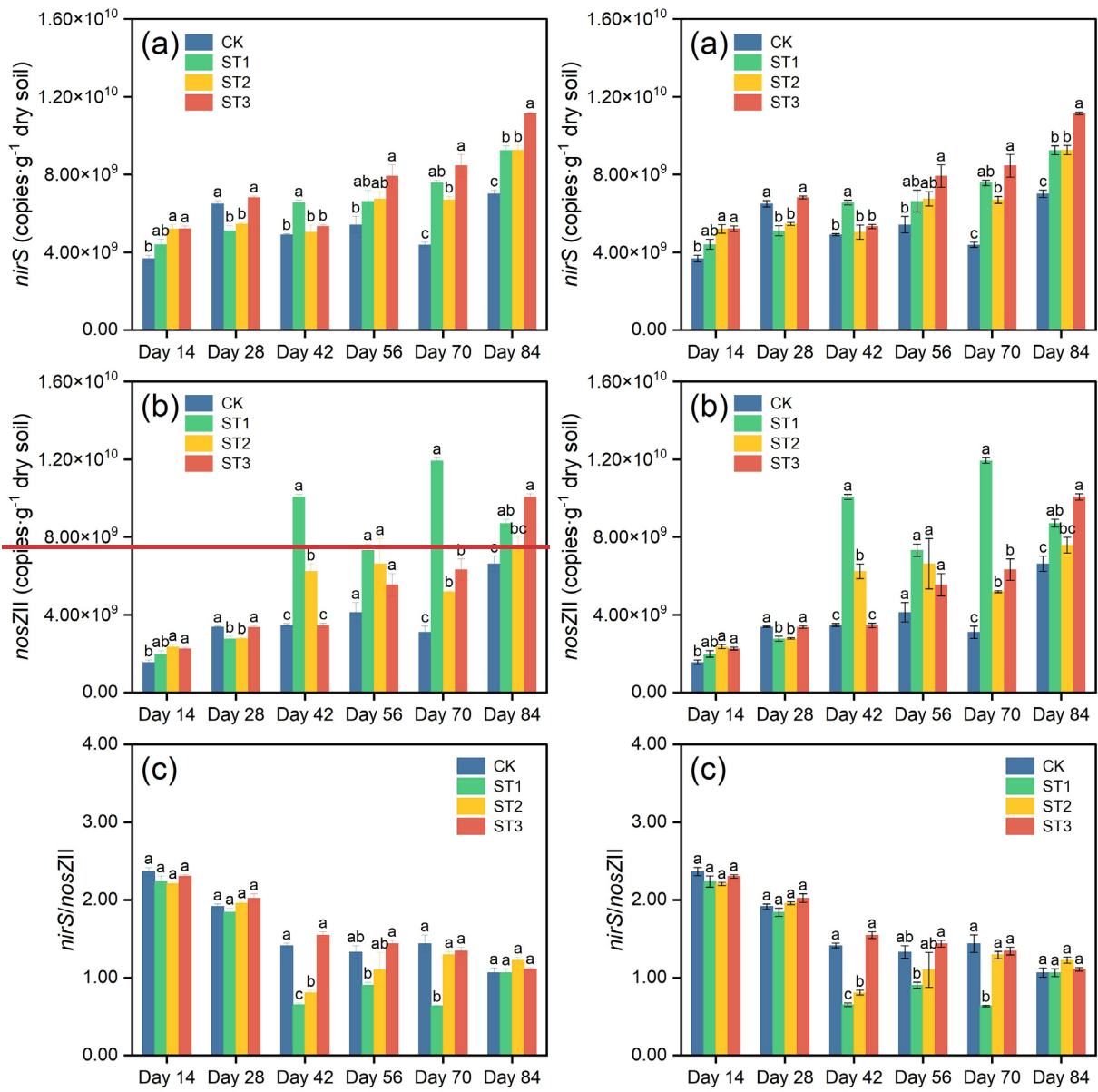


Figure 5: Dynamics of *nirS* abundance (a), *nosZII* abundance (b), and *nirS/nosZII* ratio (c) during the incubation. CK, ST1, ST2, and ST3 received 0, 50%, 100%, and 150% of the local harvested rice straw, respectively. Different lower-case letters denote significant differences between treatments ($\alpha = 0.05$). Data are given in average $\pm$ standard error ($n = 3$). Days 14 and 28 correspond to the flooding period, days 42 and 56 to the early drained period, and days 70 and 84 to the late drained period, respectively.

In general, the dynamics of *nosZII* abundance during the incubation were similar to the variations of *nirS* abundance (Fig.5b). Interestingly, the abundance of *nosZII* in ST1 was the highest, which was significantly higher than the second highest treatment (ST3) during drying, especially during the peaking time of N_2O flux on day 70. In contrast, the abundance differences of *nosZII* among treatments (Fig. S2b) were far less pronounced than those of *nosZII* (Fig. S2). When concerning the abundance ratio of *nirS/nosZII* during drying, ST1 displayed the lowest average ratio of 0.81, while the average ratios of other treatments were higher, ranging from 1.11 to 1.36 (Fig.5c).

4 Discussion

Rice straw is an important organic resource and has been used to improve soil productivity (Huo et al., 2024; Zhou et al., 2024), which was also observed in this study. Since rice straw return may also exacerbate GHG emissions from paddy fields (Shi et al., 2023; He et al., 2024), which could offset the benefits of rice straw returning to paddy systems, it is urgent to investigate the feasibility of straw incorporation in relation to soil fertility improvement and GHG emission control. Soil fertility is the capability of soil to continuously supply water, nutrients, air, and suitable thermal conditions for plant growth, which can be reflected by soil physical, chemical, and microbial characteristics (Theresa et al., 2026). We found that soil BD was negatively related to the straw incorporation rate, and soil CEC and soil available nutrient concentrations were positively related to the rate. The results strongly suggested that five-year rice straw incorporation improved soil fertility by increasing soil aeration, soil cation holding capacity, and nutrient supply ability, consistent with previous studies (Xing et al., 2025). In this study, the least amount of straw incorporation (ST1) generated the lowest GWP among straw-amended treatments. If this treatment can also lead to the elevation of soil fertility, it would support our hypothesis. The results showed that although the soil physicochemical properties of ST1 were not significantly different from those of CK, the characteristics were systematically improved. For instance, BD decreased by 2.76%, and TC and AvK increased by 4.19% and 38.52%, respectively. It is obvious that after five years of rice straw incorporation under ST1, soil porosity, soil organic matter, and nutrient supply ability all increased, indicating its soil fertility was improved and an improving trend in soil fertility was evident from the decreased BD and increased soil nutrient contents, and this trend was supported by ST2 and ST3 treatments. More importantly, ST1 treatment did not cause a significant increase in GWP compared to CK. It was observed that the cumulative CO_2 and CH_4 emissions of ST1 were significantly higher than those of CK, but the cumulative N_2O emission was significantly lower. Since the warming potentials over a 100-year horizon of CO_2 , CH_4 , and N_2O are 1, 27.9, and 273, respectively (Forster et al., 2021), the major contributor to the low GWP under ST1 would be the significantly lower N_2O emission. The interesting point is why this happened.

344 It was detected that the CO₂ fluxes were positively related to the straw incorporation rate, and the higher input induced the
 345 higher CO₂ emission. Among straw-amended treatments, ST1 generated the lowest cumulative CO₂ flux, which was about
 346 1.7 times of CK. This would be linked to the small amount of organic substrate for the soil microbial communities and the
 347 limited growth of microbial population (Miller et al., 2008; Li et al., 2022). Although the CO₂ emission of ST1 increased
 348 significantly compared to CK, its SOC content rose simultaneously, indicating this rate of straw returning was beneficial for
 349 the elevation of SOC.

350 We detected two N₂O flux peaks during the incubation: one occurred after N fertilization under flooding and the other
 351 appeared within drying, which were consistent with other studies (Zhou et al., 2020b; Senbayram et al., 2022; Li et al.,
 352 2025b). We found that only CK emitted considerable N₂O while the emissions from straw-amended treatments were
 353 markedly lower at the first peak. This would be partially due to the competition of soil microorganisms for soil available N.
 354 Generally, organic materials incorporated into paddy soil will stimulate mass propagation of soil microorganisms, and these
 355 fast reproducing microbial communities will compete for soil free N as their nutrient (Cucu et al., 2014; L'espérance et al.,
 356 2024; Zhang et al., 2025). On the contrary, the N fertilizer in CK could be an available N source for nitrification and
 357 denitrification, leading to a substantial N₂O emission. However, during drying, oxygen continuously penetrated the soil
 358 profile, and abundant available N was released mainly through the mineralization of soil organic matter (Chiriac et al., 2025).
 359 Organic matter also contributed more electron donors to denitrification when the soil moisture declined (Yi et al., 2022).
 360 These means if a soil contains higher SOC, it would produce more mineral N as the substrate and provide more electron
 361 donors for N₂O production and reduction (Yi et al., 2022; Chiriac et al., 2025). Although ST1 contained slightly higher SOC
 362 than CK, there was no clear difference in N₂O fluxes between them during drying. The reasons would be as follows. N₂O
 363 emission largely relies on N₂O production ability and consumption activity, which are driven by the related functional
 364 microorganisms (Hu et al., 2015; He et al., 2025). Among these microorganisms, the denitrifiers of *nirS*-type and *nosZII*-
 365 type communities were indicated as the more active microbial groups in paddy soils (Jones et al., 2013; Wei et al., 2015; Jin
 366 et al., 2020; Chen et al., 2024; Yang et al., 2024). Among all treatments, ST1 contained a relatively low abundance of *nirS*
 367 and the highest *nosZII* copy number, as well as the lowest *nirS/nosZII* ratio. These results strongly suggested that ST1 would
 368 possess relatively low N₂O production but high N₂O transformation activities. As a result, the N₂O emission from ST1 was
 369 significantly lower than that from CK.

370 Applying organic materials to paddy fields often intensified CH₄ flux from soils (Han et al., 2023; Song et al., 2024; Qin et
 371 al., 2025). Similar results were also observed in this study, and the CH₄ emission was significantly and positively related to
 372 the straw input rate. Interestingly, the cumulative CH₄ emission from ST1 treatment remained low, whereas ST2 and ST3
 373 sharply elevated the emissions to about 5 and 9 times that of ST1, respectively. CH₄ emission depends on CH₄ production
 374 and consumption, which are driven by methanogens and methanotrophs (Conrad, 2020; Nwokolo and Enebe, 2025). The
 375 population size of *mcrA* in ST1 was clearly higher than that in CK but significantly lower than ST2 and ST3, indicating that
 376 the CH₄ production capacity of ST1 would be higher than that of CK but lower than the treatments receiving higher amounts
 377 of straw. Another important aspect in determining CH₄ flux is the soil CH₄ consumption capability (Conrad, 2020; Nwokolo

and Enebe, 2025). Soil CH₄ consumption would be strongly linked to soil CH₄ holding capacity (Ariani et al., 2022), as the retained CH₄ can be oxidized by methanotrophs. Once CH₄ producing ability is over the soil CH₄ holding capacity, the extra CH₄ would be emitted into the atmosphere. We observed that the soil CH₄ holding capacity was about 190 mmol·mol⁻¹ under the anaerobic environment, and below this level CH₄ emission was almost undetectable. It was detected that the CH₄ concentrations of ST2 and ST3 were clearly over the holding capacity, and CH₄ emissions notably occurred. In contrast, the soil CH₄ concentrations of ST1 fluctuated around 200 mmol·mol⁻¹ under anaerobic conditions, leading to the minimal CH₄ emission among straw-amended treatments. Moreover, the microbial gene abundances in this study were at the higher end of the ranges reported in the literature (Ma et al., 2012; Zhou et al., 2020b; Wang et al., 2022; Yi et al., 2024; Cai et al., 2025; Zheng et al., 2026). This could be attributed to the differences in soil properties among studies and to straw incorporation that provided substrates for microbial growth.

5 Conclusion

~~Rice straw could be incorporated into paddy soil at a suitable amount. In general, rice straw incorporation could improve soil nutrient supply ability for crops and simultaneously intensify GHG emissions. However, we revealed that when the straw was incorporated at a suitable amount, i.e., 50% of the local rice straw yield, GHG emissions remained at low levels that did not cause a significant increase in GWP compared to CK, and soil fertility was well maintained. Compared to no straw input, it could not only improve soil fertility, but also avoid significantly increasing GWP.~~ The major contributors to this consequence were strongly linked to the highest *nosZII* copy number and the lowest *nirS/nosZII* ratio, resulting in a significantly lower N₂O cumulative emission than CK. ~~and~~ The CH₄ producing ability under ST1 fluctuated around the soil CH₄ holding capacity-, leading to and most of the produced CH₄ was-being consumed by ~~soil~~ methanotrophs in soil and minimal CH₄ being emitted into the atmosphere. These findings offer scientific guidance for rational straw use, sustainable rice farming, and eco-friendly paddy management. Because different soils possess various soil properties, their CH₄ holding capacities and nitrogen transformation processes would be different. Therefore, the field tests with different soil types are necessary.

Data availability

Data will be made available on reasonable request.

Supplement link

The link to the supplement will be included by Copernicus, if applicable.

405 **Author contributions**

406 **MZ:** Conceptualization, Data Curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing -
407 Original Draft, Writing - Review & Editing. **RL:** Data Curation, Formal analysis. **WZ:** Conceptualization, Resources,
408 Supervision, Writing - Review & Editing. **CF:** Resources. **SGC** and **AT:** Writing - Review & Editing. **BZ:**
409 Conceptualization, Resources, Supervision, Writing - Review & Editing. **WW:** Conceptualization, Methodology, Resources,
410 Supervision, Validation, Writing - Original Draft, Writing - Review & Editing. **RS:** Conceptualization, Funding acquisition,
411 Resources, Supervision, Writing - Original Draft, Writing - Review & Editing.

412 **Competing interests**

413 The authors declare that they have no conflict of interest.

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427 **Review statement**

428 The review statement will be added by Copernicus Publications listing the handling editor as well as all contributing referees
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